Primary Evaluator

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Date: 10/18/2006

Approved by

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Date: 10/18/2006

STUDY REPORTS:

46165901 Hamilton, L. (2003) Analytical Method for the Determination of Residues of CGA-21947 and Its Metabolites, CGA-232499, CGA-249287, CGA-263208, and NOA-422054 in Crops by High Performance Liquid Chromatography With Mass Spectrometry. Project Number: 2213/01. Unpublished study prepared by Syngenta Crop Protection, Inc. 198 p.

EXECUTIVE SUMMARY:

Syngenta Crop Protection, Inc. has submitted an analytical method (Analytical Method 2213-01) for the determination of residues of cyprodinil and its metabolites CGA-232499, CGA-249287, CGA-263208, and NOA-422054 in/on various plant commodities. The method is entitled: "*Analytical Method for the Determination of Residues of CGA-21947 and Its Metabolites, CGA-232499, CGA-249287, CGA-263208, and NOA-422054 in Crops by High Performance Liquid Chromatography With Mass Spectrometry*".

Analytical Method 2213-01 was used to determine residues of cyprodinil and its metabolites in/on the following commodities associated with the studies submitted in conjunction with DP No. 326346: lettuce; garden beet tops and roots; wheat forage, hay, straw, and grain from the field rotational crop and storage stability studies.

For Analytical Method 2205-01, samples are extracted by shaking with methanol/water (80/20, v/v). An aliquot of the extract is evaporated until only the aqueous phase remains. The aqueous fraction is cleaned using a C18 solid-phase extraction (SPE) cartridge. The analytes are eluted from the SPE cartridge with acetonitrile/0.1 M sodium hydroxide (70/30, v/v). Note: SPE cleanup is used for the analysis of cyprodinil and CGA-232449, CGA-249284, and NOA-422054 only. Residues of CGA-263208 are determined without SPE clean-up of the initial aqueous phase. The extract (containing CGA-263208) is reduced to the aqueous phase. Both extracts are diluted to final volume with 0.1% ammonium acetate in water. The analytes are quantified by analysis utilizing high-performance liquid chromatography (HPLC) with mass-spectrometric (MS) detection. For all commodities, the validated limit of quantitation (LOQ) was 0.05 ppm for all analytes. The limit of detection (LOD) was reported to be 0.01 ng.

Percent recoveries and standards of deviation indicate acceptable accuracy/precision at the LOQ and up to 10 times the LOQ for residues of cyprodinil and its metabolites in/on lettuce; beet tops and roots; wheat forage, hay, straw, and grain. The fortification levels and samples used in method validation and in conjunction with the concurrent fortification data are adequate to bracket expected residue levels. Concurrent method recovery data were included with the rotational crop and storage stability studies submitted in conjunction with DP Number 326346. Concurrent method validation data were submitted for the following commodities: lettuce; garden beet tops and roots; wheat forage, hay, straw, and grain.

No confirmatory method data were included with this method. However, the method includes instructions for analysis utilizing an ultraviolet (UV) detector and further states that residues of cyprodinil may be confirmed via use of a MS/MS detection system.

The extraction solvents used in Analytical Method 2213-01 are identical to those used in the enforcement method for tolerances for cyprodinil in/on plant commodities (Analytical Method AG-631B, MRID 44621202). Analytical Method AG-631B has been successfully radiovalidated (Memo, 2/20/98, G. Kramer, D233182).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The submitted analytical method data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Number 326346.

COMPLIANCE:

Signed and dated Good Laboratory Practices (GLP), Quality Assurance, and Data Confidentiality statements were provided. Minor deviations from regulatory requirements were reported. However, these deviations did not have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Cyprodinil is an anilino-pyrimidine fungicide used to control various plant diseases. Permanent tolerances are established (40 CFR 180.532) for residues of cyprodinil in/on a variety of raw agricultural commodities (RACs). Additionally, time-limited tolerances have been established for residues in/on strawberries and onion, dry bulb (0.60 ppm) and onion, green (4.0). These tolerances are set to expire on 12/31/2007.

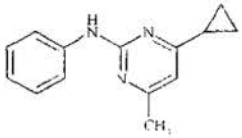
TABLE A.1. Nomenclature of Test Compound.	
Compound	
Common name	Cyprodinil
Company experimental names	CGA-219417
IUPAC name	4-cyclopropyl-6 methyl-*N*-phenylpyrimidin-2-amine
CAS name	4-cyclopropyl-6-methyl-*N*-phenyl-2-pyrimidinamine
CAS #	121552-61-2
End-use products/EP	SWITCH 62.5WG, EPA Reg. No. 100-953 (containing 37.5% cyprodinil and 25.0% fludioxonil)

TABLE A.2. Physicochemical Properties of Technical Grade Cyprodinil.		
Parameter	Value	Reference
Melting point	75.9 C	MRID 43709003
pH	9.5 at 25 C (1% aqueous dispersion)	MRID 43709003
Density	1.21 g/cm^3 at 20 C	MRID 43709003
Water solubility	16 mg/L in water, pH 7.6 (pure water) at 25 C 20 mg/L in pH 5 buffer at 25 C 13 mg/L in pH 7 buffer at 25 C 15 mg/L in pH 9 buffer at 25 C	MRID 46584601
Solvent solubility	ethanol: 160 g/L at 25 C acetone: 610 g/L at 25 C toluene: 460 g/L at 25 C *n*-octanol: 160 g/L at 25 C *n*-hexane: 30 g/L at 25 C	MRID 46584601
Vapor pressure	3.8 x 10^{-6} mm Hg at 25 C	MRID 43709003
Dissociation constant, pK$_a$	pKa = 4.44 at 25 C	MRID 43709003
Octanol/water partition coefficient, Log(K$_{OW}$)	3.9 @ pH = 5 4.0 @ pH = 7 4.0 @ pH = 9	Fact Sheet, 4/6/98
UV/visible absorption spectrum	NA[1]	

[1] NA = information not available to the reviewer.

B. MATERIALS AND METHODS

B.1. Data-Gathering Methods

A data gathering method (referred to as Analytical Method 2213-01) for the determination of cyprodinil and its metabolites CGA-232449, CGA-249287, CGA-263208, and NOA-422054 in various crops was submitted. This method was used in conjunction with storage stability and field rotational crop studies associated with DP Number 326346. The submitted method is entitled: "*Analytical Method for the Determination of Residues of CGA-21947 and Its Metabolites, CGA-232499, CGA-249287, CGA-263208, and NOA-422054 in Crops by High Performance Liquid Chromatography With Mass Spectrometry.*"

Analytical Method 2213-01 was used to determine residues of cyprodinil and its metabolites in/on the following commodities associated with the studies submitted in conjunction with DP Number 326346: lettuce; garden beet tops and roots; wheat forage, hay, straw, and grain.

B.1.1. Principle of the Methods:

For Analytical Method 2205-01, samples are extracted by shaking with methanol/water (80/20, v/v). An aliquot of the extract is evaporated until only the aqueous phase remains. The aqueous fraction is cleaned using a C18 SPE cartridge. The analytes are eluted from the SPE cartridge with acetonitrile/0.1 M sodium hydroxide (70/30, v/v). Note: SPE cleanup is used for the analysis of cyprodinil and CGA-232449, CGA-249284, and NOA-422054 only. Residues of CGA-263208 are determined without SPE clean-up of the initial aqueous phase. The extract (containing CGA-263208) is reduced to the aqueous phase. Both extracts are diluted to final volume with 0.1% ammonium acetate in water. The analytes are quantified by analysis utilizing HPLC/MS.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Residues of Cyprodinil and Its Metabolites in Plant Commodities.	
Method ID	Analytical Method No. 2213-01
Analytes	Cyprodinil and its metabolites (CGA-232499, CGA-249287, CGA-263208, and NOA-422054)
Matrices	Lettuce; beet tops and roots; wheat forage, hay, straw, and grain; and onions
Extraction solvent/technique	All commodities are extracted on a mechanical shaker with methanol/water (80/20, v/v).
Cleanup strategies	The extracts are filtered and concentrated to aqueous. An aliquot of the aqueous phase of the extract is applied to a C18 SPE cartridge. The cartridge is washed with methanol followed by water. Residues are eluted from the cartridge with acetonitrile/0.01M sodium hydroxide (70/30, v/v). The eluate is diluted to final volume with 0.1% ammonium acetate in water. Note: SPE cleanup is used for the analysis of CGA-219417 (cyprodinil) and CGA-232449, CGA-249284, and NOA-422054 only. Residues of CGA-263208 are determined without SPE clean-up of the initial aqueous phase. The extract (containing CGA-263208) is reduced to the aqueous phase and diluted to final volume with 0.1% ammonium acetate in water.
Instrument/Detector	HPLC utilizing a reverse-phase column and a gradient mobile phase of 0.1% ammonium acetate in water and 0.1% ammonium acetate in acetonitrile, with mass-spectrometric detection using electrospray ionization operating in the positive ion mode with multiple reaction monitoring. The ions monitored for the various analytes are: 226 amu (cyprodinil), 136 amu (CGA-263208), 150 amu (CGA-249287), 166 amu (NOA-422054), and 242 amu (CGA-232449).
Standardization method	Calibration curve of detector response to amount injected utilizing external standard solutions in the range of 0.0005 to 0.025 ng/uL.
Stability of std solutions	Standard solutions may be made individually or combined as appropriate. Solutions are prepared in acetonitrile. Standard solutions are to be stored refrigerated (temperature not specified) and protected from light. The stability of standard solutions was not evaluated in the submitted study.
Retention times	Cyprodinil : ~17 min CGA-232449: ~14.5 min CGA-249287: ~10 min CGA-263208: ~7.0 min NAO-422054: ~7.5 min

B.2. Enforcement Methods

Analytical Method 2213-01 is not proposed for enforcement of tolerances.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Methods

TABLE C.1.1.1. Recovery Results for Method Validation of Analytical Method 2213-01 for the Determination of Cyprodinil and Its metabolites in/on Plant Commodities.

Matrix	Analyte	Fortification Level (ppm)	Recovery (%)	Mean Recovery (Std. Dev.)
Wheat Forage	Cyprodinil	0.05	92, 111	98% (8.5%)
		0.10	100	
		0.20	89	
	CGA-232449	0.05	117	92% (18%)
		0.10	78	
		0.20	82	
	CGA-249287	0.05	82	82% (2.1%)
		0.10	85	
		0.20	80	
	CGA-263208	0.05	75, 78	74% (5.0%)
		0.10	76	
		0.20	*65*	
	NOA-422054	0.05	118, 84	99% (19%)
		0.10	117	
		0.20	75	
Wheat Hay	Cyprodinil	0.05	84, 72	77% (9.2%)
		0.20	88	
		0.50	*65*	
	CGA-232449	0.05	84, 74	87% (9.7%)
		0.20	101	
		0.50	87	
	CGA-249287	0.05	76, *69*	82% (12%)
		0.20	102	
		0.50	78	
	CGA-263208	0.05	81, *62*	75% (7.6%)
		0.20	75	
		0.50	80	
	NOA-422054	0.05	79	88% (8.3%)
		0.20	99	
		0.50	86	
Wheat Straw	Cyprodinil	0.05	74, *64*	73% (8.5%)
		0.10	86	
		0.50	67	
	CGA-232449	0.05	86, 81	88% (11%)
		0.10	107	
		0.50	79	
	CGA-249287	0.05	93, 79	88% (11%)
		0.10	77	
		0.50	104	
	CGA-263208	0.05	82, 94	82% (7.4%)
		0.10	74	
		0.50	79	
	NOA-422054	0.05	101, 73	83% (11%)
		0.10	82	
		0.50	77	
Wheat Grain	Cyprodinil	0.05	95, *66*	89% (25%)
		0.10	128	

TABLE C.1.1.1. Recovery Results for Method Validation of Analytical Method 2213-01 for the Determination of Cyprodinil and Its metabolites in/on Plant Commodities.

Matrix	Analyte	Fortification Level (ppm)	Recovery (%)	Mean Recovery (Std. Dev.)
		0.20	67	
	CGA-232449	0.05	103, 104	98% (7.2%)
		0.10	98	
		0.20	86	
	CGA-249287	0.05	75, 82	80% (6.4%)
		0.10	74	
		0.20	90	
	CGA-263208	0.05	71, 83	77% (4.8%)
		0.10	79	
		0.20	73	
	NOA-422054	0.05	112, 87	91% (14%)
		0.10	94	
		0.20	72	
Lettuce	Cyprodinil	0.05	103, 73	98% (20%)
		0.10	*128*	
		0.25	88	
	CGA-232449	0.05	107, 81	98% (13%)
		0.10	114	
		0.25	91	
	CGA-249287	0.05	87, 111	95% (9.5%)
		0.10	89	
		0.25	92	
	CGA-263208	0.05	82, 90	85% (8.7%)
		0.10	72	
		0.25	95	
	NOA-422054	0.05	106, 102	108% (11%)
		0.10	*126*	
		0.25	96	
Garden Beet Tops	Cyprodinil	0.05	80, 73	80% (10%)
		0.20	97	
		0.50	71	
	CGA-232449	0.05	70, 99	89% (14%)
		0.20	106	
		0.50	81	
	CGA-249287	0.05	98, 71	86% (13%)
		0.20	101	
		0.50	75	
	CGA-263208	0.05	89, 91	90% (4.7%)
		0.20	84	
		0.50	97	
	NOA-422054	0.05	89, 71	82% (8.3%)
		0.20	91	
		0.50	77	
Garden Beet Roots	Cyprodinil	0.05	79, *68*	81% (9.2%)
		0.10	94	
		0.50	81	
	CGA-232449	0.05	94, 78	94%

TABLE C.1.1.1. Recovery Results for Method Validation of Analytical Method 2213-01 for the Determination of Cyprodinil and Its metabolites in/on Plant Commodities.

Matrix	Analyte	Fortification Level (ppm)	Recovery (%)	Mean Recovery (Std. Dev.)
		0.10	98	(9.6%)
		0.50	104	
	CGA-249287	0.05	81, 86	89%
		0.10	77	(13%)
		0.50	111	
	CGA-263208	0.05	72, 92	82%
		0.10	84	(7.4%)
		0.50	78	
	NOA-422054	0.05	92, 96	97%
		0.10	108	(6.4%)
		0.50	93	

The fortification levels and samples used in the method validation are adequate to bracket expected residue levels. Concurrent method recovery data, bracketing the reported residue levels, were included with the storage stability and rotational crop studies submitted in conjunction with DP Number 326346. Concurrent method validation data were submitted for the following commodities: lettuce; garden beet tops and roots; wheat forage, hay, straw, and grain.

No confirmatory method was included with this method. However, the method includes instructions for analysis utilizing a UV detector and further states that residues of cyprodinil may be confirmed via use of a MS/MS detection system.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Methods Used for the Quantitation of Residues of Cyprodinil and Its Metabolites.	
Method ID	Analytical Method 2213-01
Analytes	Cyprodinil and its metabolites (CGA-232499, CGA-249287, CGA-263208, and NOA-422054)
Equipment ID	Waters 2690 High-Performance Liquid Chromatograph or equivalent with autosampler, quaternary gradient elution pump and mass spectrometry detection.
Limit of quantitation (LOQ)	LOQ = 0.05 ppm for all analytes in/on lettuce; beet tops and roots; wheat forage, hay, straw, and grain.
Limit of detection (LOD)	LOD = 0.01 ng for all analytes.
Accuracy/Precision	Percent recoveries and standard of deviation indicate acceptable accuracy/precision at the LOQ and up to 10 times the LOQ for residues of cyprodinil and its metabolites in/on on lettuce; beet tops and roots; wheat forage, hay, straw, and grain. Recovery ranges, average recovery and standards of deviation, respectively, from these matrices were as follows: Wheat forage: cyprodinil, 89-111%, 98%, (8.5), CGA-232449, 78-117%, 92% (18); CGA-249287, 80-85%, 82% (2.1); CGA-263208, 65-78%, 74% (5.0); NOA-422054, 75-118%, 99% (19) Wheat hay: cyprodinil, 65-88%, 77%, (9.2), CGA-232449, 84-101%, 87% (9.7); CGA-249287, 69-102%, 82%, (12); CGA-263208, 62-81%, 75%, (7.6); NOA-422054, 79-99%, 88% (8.3) Wheat straw: cyprodinil, 64-86%, 73% (8.5), CGA-232449, 79-107%, 88%, (11); CGA-249287, 77-104%, 88%, (11); CGA-263208, 74-94%, 82% (7.4); NOA-422054, 73-101%, 83% (11) Wheat grain: cyprodinil, 66-128%, 89%, (25), CGA-232449, 86-104%, 98%, (7.2); CGA-249287, 74-90%, 80%, (6.4); CGA-263208, 71-83%, 77% (4.8); NOA-422054, 72-112%, 91%, (14) Lettuce: cyprodinil, 73-128%, 98%, (20), CGA-232449, 81-114%, 98%, (13); CGA-249287, 87-111%, 95%, (9.5); CGA-263208, 72-95%, 85%, (8.7); NOA-422054, 96-126%, 108% (11) Beet Tops: cyprodinil, 71-97%, 80%, (10), CGA-232449, 70-106%, 89%, (14); CGA-249287, 71-101%, 86% (13); CGA-263208, 84-97%, 90%, (4.7); NOA-422054, 71-91%, 82% (8.3) Beet roots: cyprodinil, 68-94%, 81%, (9.2), CGA-232449, 78-104%, 94% (9.6); CGA-249287, 81-111%, 89% (13); CGA-263208, 72-92%, 82%, (7.4); NOA-422054, 92-108%, 97% (6.4)
Reliability of the Method/ [ILV]	An independent laboratory validation (ILV) was not submitted.
Linearity	The detector response was linear (r^2 = 0.99) in the range of 0.005 ng to 0.25 ng.
Specificity	The control chromatograms generally have no peaks above the chromatographic background near the analyte peak of interest. Peaks were well defined and symmetrical.

The extraction solvents used in Analytical Method 2213-01 are identical to those used in the enforcement method for tolerances for cyprodinil in/on plant commodities (Analytical Method AG-631B, MRID 44621202). Analytical Method AG-631B has been successfully radiovalidated (Memo, 2/20/98, G. Kramer, D233182).

C.2. Enforcement Method

The submitted method is not proposed for enforcement of tolerances.

C.3. Independent Laboratory Validation (ILV)

ILV data for Analytical Method 2213-01 were not submitted.

D. CONCLUSION

Adequate method validation data have been submitted for Analytical Method 2213-01 for the determination of residues of cyprodinil and its metabolites (CGA-232499, CGA-249287, CGA-263208, and NOA-422054) in/on lettuce, garden beet tops and roots; and wheat forage, hay, straw, and grain. Recovery ranges, average recoveries, and standards of deviation, respectively, from these matrices were as follows:

Wheat forage: cyprodinil, 89-111%, 98%, (8.5), CGA-232449, 78-117%, 92% (18);
CGA-249287, 80-85%, 82% (2.1); CGA-263208, 65-78%, 74% (5.0); NOA-422054, 75-118%, 99% (19)

Wheat hay: cyprodinil, 65-88%, 77%, (9.2), CGA-232449, 84-101%, 87% (9.7);
CGA-249287, 69-102%, 82%, (12); CGA-263208, 62-81%, 75%, (7.6); NOA-422054, 79-99%, 88% (8.3)

Wheat straw: cyprodinil, 64-86%, 73% (8.5), CGA-232449, 79-107%, 88%, (11);
CGA-249287, 77-104%, 88%, (11); CGA-263208, 74-94%, 82% (7.4); NOA-422054, 73-101%, 83% (11)

Wheat grain: cyprodinil, 66-128%, 89%, (25), CGA-232449, 86-104%, 98%, (7.2);
CGA-249287, 74-90%, 80%, (6.4); CGA-263208, 71-83%, 77% (4.8); NOA-422054, 72-112%, 91%, (14)

Lettuce: cyprodinil, 73-128%, 98%, (20), CGA-232449, 81-114%, 98%, (13);
CGA-249287, 87-111%, 95%, (9.5); CGA-263208, 72-95%, 85%, (8.7);
NOA-422054, 96-126%, 108% (11)

Beet Tops: cyprodinil, 71-97%, 80%, (10), CGA-232449, 70-106%, 89%, (14);
CGA-249287, 71-101%, 86% (13); CGA-263208, 84-97%, 90%, (4.7); NOA-422054, 71-91%, 82% (8.3)

Beet roots: cyprodinil, 68-94%, 81%, (9.2), CGA-232449, 78-104%, 94% (9.6);
CGA-249287, 81-111%, 89% (13); CGA-263208, 72-92%, 82%, (7.4); NOA-422054, 92-108%, 97% (6.4)

The petitioner has not proposed Analytical Method 2213-01 for enforcement. The extraction solvents used in Analytical Method 2213-01 are identical to those used in the enforcement method for tolerances for cyprodinil in/on plant commodities (Analytical Method AG-631B, MRID 44621202). Analytical Method AG-631B has been successfully radiovalidated (Memo, 2/20/98, G. Kramer, D233182). ILV data for this method were not submitted.

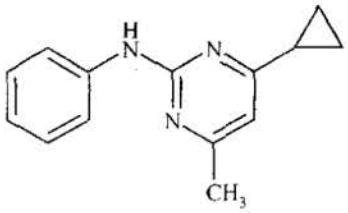
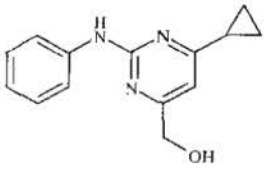
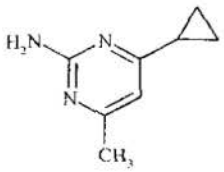
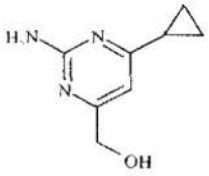
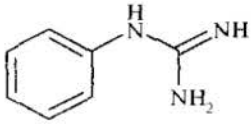
E. REFERENCES

Memo, 2/20/98, G. Kramer, D233182.

F. DOCUMENT TRACKING

RDI: RAB1 Chem. Team: 09/20/2006.
Petition Number: 8E05012
DP Number: 326346
PC Code: 288202

Template Version September 2003

Attachment 1: Chemical Names and Structures of Cyprodinil and its Metabolites of Toxicological Concern in Rotational Crops.	
Chemical Structure	**Chemical Structure**
Cyprodinil (CGA-219417) 4-cyclopropyl-6-methyl-*N*-phenyl-2-pyrimidinamine	
CGA-232449 6-cyclopropyl-2-(phenylamino)-4-pyrimidinemethanol	
CGA-249287 4-cyclopropyl-6-methyl-2-pyrimidinamine	
NOA-422054 4-cyclopropyl-6-hydroxymethyl-2-pyrimidinamine	
CGA-263208 phenylguanidine	

13544

R133358

Chemical: Cyprodinil

PC Code:
288202

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Memo Date: 10/18/2006
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Accession #: 412-07-0024

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11/17/2006